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MICROCALORIMETRIC STUDY OF BACTERIAL GROWTH

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ABSTRACT

The fundamental growth thermograms of *Escherichia coli, Staphylococcus aureus, Salmonella typhosa, Salmonella chaleraeuis* and *Shigella flexneri* have been determined by the microcalorimetric method. These perfect thermogram curves reflect the changes of bacterial growth patterns (including the lag phase of growth, log growth, stationary phase and decline phase of growth). In our experiments, highly characteristic and reproducible growth patterns are observed under the same conditions; therefore, one can use these thermograms as a "finger print" to identify bacteria.

Further research on these thermograms has enabled us to calculate the multiplication rate constant and activation energy of bacterial growth. Obviously, these thermogram curves contain ample information which is very significant for studies in microbiological, bio-thermokinetic and clinical fields.

INTRODUCTION

In any living system the various metabolic events occurring within the cells are all reactions producing heat. Thus, by monitoring the heat effects with sufficient sensitive calorimeters, we can study the metabolic processes of living cells. By continuous measurement of the heat effects of the growing cells with a calorimeter, one can see that the time dependence of the observed thermal effect (the thermogenesis curve) reflects time dependent changes in growth patterns. Thus the calorimeter is a powerful tool in observing living cells, in that it can provide information on their metabolism. It has recently been demonstrated that calorimetric methods can be used for fundamental growth studies of bacteria [1,2]. For example, Boling et al. examined growth patterns of *Enterobacter aerogenes* and *Klebsiella*.

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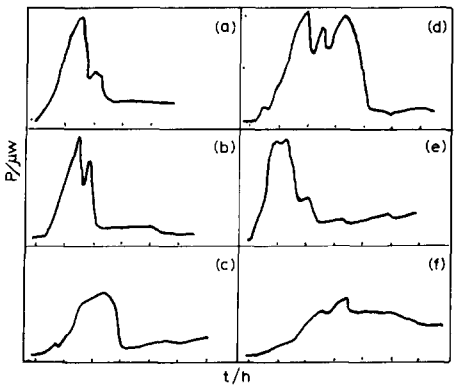

Fig. 1. Thermograms of bacterial growth determined by boiling: (a) *Enterobacter aerogenes*, (b) *Klebsiella*, (c) *Proteus vulgaris*, (d) *Enterobacter cloacae*, (e) *Escherichia coli*, (f) *Proteus rettgeri*.

The instrument used by those workers was a 50-channel batch calorimeter. The bacteria were cultured in brain–heat infusion media at 37°C. The thermograms of bacterial growth were recorded and were of the type shown in Fig. 1. Apparently, highly characteristic and reproducible thermograms can be obtained under the same conditions of culture temperature, media and experimental technique, so these thermograms may be used as a "finger print" for the identification of bacteria.

In general, the law of bacterial growth contains the lag phase (as AB), log phase (BC), stationary phase (CD) and decline phase of growth (DE). So the thermogram of bacterial growth should be of the shape shown in Fig. 2. Some of the thermograms curves determined by Boling et al. [3] are incomplete because the lag phase and partial log phase of growth are missing. The reason for this is that those experiments were done using a batch calorimeter, and this usually takes a long time (1–2 h) to reach equilibrium. So these thermograms did not start from the lag phase of growth.

In this work, we used an LKB 2277 Bioactivity Monitor in determining the thermograms of bacterial growth. The bacterium sample was cultured in

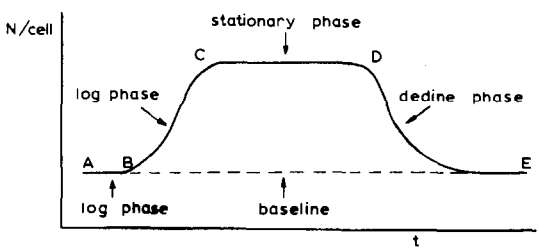

Fig. 2. Different phases of bacterial growth.

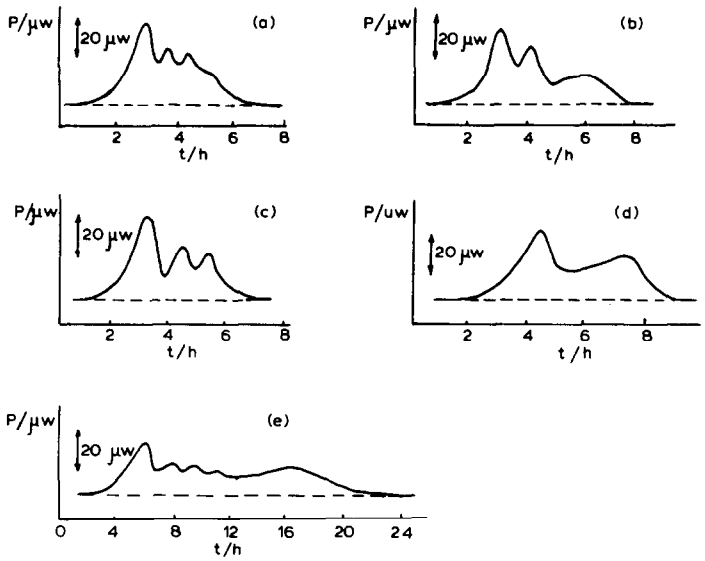

Fig. 3. Thermograms of bacteria obtained at 37 °C, (cultured in peptone): (a) *Escherichia coli*, (b) *Staphylococcus aureus*, (c) *Salmonella typhosa*, (d) *Shigella flexneri*, (e) *Salmonella chaleraeuis*.

the flow cell of the monitor system (stop pump experimental model). In these experiments, perfect thermogram curves of *Escherichia coli, Staphylococcus aureus, Salmonella typhosa, Salmonella chaleraeuis* and *Shigella flexeri* have been determined (see Fig. 3). The thermogram of each bacterium shows highly characteristic and reproducible growth patterns under the same conditions. From these thermograms curves (log phase) the multiplication rate constants (k) and the generation times (G) have been calculated and the results coincide with the literature values (determined by general biological methods).

Finally the thermograms have been determined at different temperature and the corresponding multiplication rate constants calculated (see Fig. 6 and Table 4). These results showed that the ln k, vs. T^{-1} curves are linear (correlation coefficient $r = 0.99$). So, with the help of the Arrhenius relation, the activation energies (E_a) and pre-exponential factor (Z) of bacterial growth were calculated. These results are very significant for the study of bacterial growth and metabolic processes.

INSTRUMENT AND MATERIALS

Instrument

A new type of heat-flow microcalorimeter, the LKB 2277 Bioactivity Monitor, is used in this experiment. It is designed to monitor continuously a

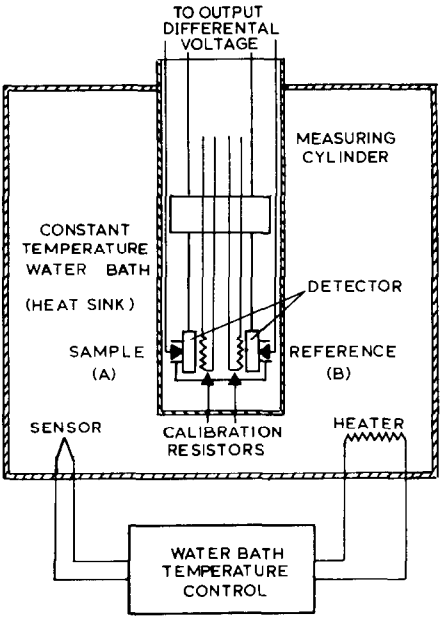

Fig. 4. Simplified operation diagram.

wide variety of processes and complex systems over the temperature range 20–80 °C. A schematic representation of the calorimetry system is shown in Fig. 4. Each measuring cylinder normally contains a sample and a reference in separate measuring cups (twin system). The heat output from the sample flows from the thermoelectric detector to the large heat sink (in close contact with the water bath). In response the detector produces a voltage which is proportional to the power output from the sample. In order to minimize the systematic errors and disturbance effects, a differential or twin detector system is used. This system is very sensitive, the detection limit is 0.15 μW and the baseline stability (over a period of 24 hours) is 0.2 μW. There are three operating modes for the LKB Bioactivity Monitor: ampoule mode, flow-through mode and flow–mix mode. In this experiment the flow-through mode has been used. The sample is pumped through the flow cell by a Microperpex pump (LKB 2132).

In the monitoring system, two precision resistors for electrical calibration are built into each measuring cylinder, one for each detector. When a known current is passed through the appropriate resistor, the detector can be calibrated easily. Other methods for calibration are suitable internally calibrated radioactive sources and chemical test reactions. Using one of these techniques, the calorimetric constant (ϵ in units of μJ cm^{-2} or μW cm^{-1}) can be determined. The time constant (τ) of this instrument is about 120 s. The performance of this instrument and the details of its construction have been previously described [4].

Materials

The following bacterial strains, were employed: (1) *Escherichia coli*, (2) *Staphylococcus aureus*, (3) *Salmonella typhosa*, (4) *Salmonella chaleraeuis* and (5) *Shigella flexeri*.

A peptone medium (pH = 7.2–7.4) was used, containing in every 6 l the following: NaCl, 30 g; K_2HPO_3, 12 g; *p*-aminobenzoic acid, 0.12 g; peptone, 30 g; sodium citrate, 72 g; $MgSO_4$, 30 g; NaOH (2N), 15 ml; tryptone, 30 g; beef extract, 18 g, and tryptose, 30 g.

All the above materials were provided by the Army Hospital of Kwangchow Military Area.

EXPERIMENT AND RESULTS

Experimental method of bacterial culture

A schematic representation of the experimental apparatus is shown in Fig. 5.

The complete cleaning and sterilization procedure for the flow tubing was as follows.

(a) Sterilized distilled water was pumped through the system for 30 min at a flow rate of 40 ml h^{-1}.

(b) 0.1N H_2SO_4 (or 0.1N HCl) was pumped through the system for 30 min at a flow rate of 40 ml h^{-1}.

(c) Alcohol solution (75%) was pumped through the system for 30 min at a flow rate of 25 ml h^{-1}.

(d) Sterilized distilled water was again pumped through the system for 30 min, at a flow rate of 40 ml h^{-1}.

Once the system was cleaned and sterilized, sterilized distilled water was pumped through the system at a flow rate of 10 ml h^{-1} and the baseline was determined. When the baseline had been obtained following a stabilization period, the bacteria sample was pumped into the flow cell at the same flow

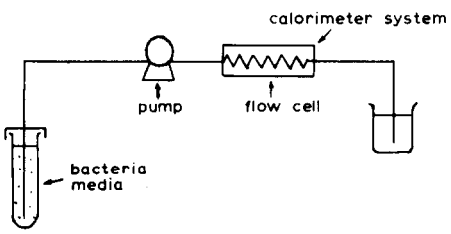

Fig. 5. Culture in flow cell schematic diagram.

rate. When the flow cell (volume 0.6 ml) was full the pump was stopped and the monitor continued to record the thermograms of bacterial growth.

Once the pen of the chart recorder had returned to the baseline and stabilized, the bacterial growth had ended. When necessary, further calibration was done after a stable baseline has been obtained.

The thermograms obtained by this experimental method are shown in Fig. 3.

Calculation of the multiplication rate constant of bacteria

In the log phase of growth, the bacterial number and culture time correspond to an exponential law [5].

If the bacterial number is n_0 at time t_0, and n_t at time t, then

$$n_t = n_0 \, e^{k(t-t_0)} \tag{1}$$

When $t_0 = 0$ (at the starting point of the log phase of growth)

$$n_t = n_0 \, e^{kt} \tag{2}$$

k is the multiplication rate constant. If the power output of each bacterium is w

$$n_t w = n_0 w \, e^{kt} \tag{3}$$

We write $W_0 = n_0 w$ and $W_t = n_t w$ giving

$$W_t = W_0 \, e^{kt} \text{ or } \ln W_t = \ln W_0 + kt \tag{4}$$

Thus the thermogenesis curve of the log phase of growth (BC) should obey eqn. (4). So in making use of the data $\ln W_t$ and t taken from the BC curve to fit a linear equation, one can obtain the rate constant (k).

TABLE 1

Data of W_t–t for experiment 3 of *Escherichia coli*

t (min)	W_t (μW)	$\ln W_t$
12	1.488	0.3975
22	2.480	0.9803
32	3.968	1.378
42	6.349	1.848
52	9.425	2.243
62	12.90	2.557
72	19.84	2.988
82	27.28	3.306
91	37.20	3.616

$\ln W_t = 0.04798 + 0.04028t$.
$k = 0.04028 \text{ min}^{-1}$ $r = 0.9971$.

TABLE 2

Multiplication rate constants of *Escherichia coli* at 37 °C

Experiment number	k (min^{-1})	r
1	0.03846	0.9991
2	0.03736	0.9955
3	0.04028	0.9971
4	0.03793	0.9991
5	0.03638	0.9979
6	0.03993	0.9981
7	0.04219	0.9970
8	0.03887	0.9982
9	0.04178	0.9987
10	0.04524	0.9942
11	0.03810	0.9904

Average $k = 0.03968 \pm 0.00257$.

For example, the data of experiment 3 on *Escherichia coli* is shown in Table 1 and its corresponding equation is as follows:

$$\ln W_t = 0.04798 + 0.04028\, t \tag{5}$$

with correlation coefficient $r = 0.9971$. This gives a rate constant $k = 0.04028$ min^{-1}.

For *Escherichia coli* 11 experiments have been done at 37 °C, the rate constants of all experiments are shown in Table 2. These results are approximately constant with an average value $k = 0.03968 \pm 0.00257$ min^{-1}. The rate constants for *Staphylococcus aureus, Salmonella typhosa, Salmonella chaleraeuis* and *Shigella flexneri* are shown in Table 3. They were determined by the same experimental method.

Activation energy of bacterial growth

The thermograms of *Escherichia coli* and *Shigella flexneri* have been determined at different temperature with same experimental method. Simi-

TABLE 3

Bacterial mutliplication rate constants at 37 °C

Bacteria sample	Rate constant k (min^{-1})			Average
	1	2	3	
Staphylococcus aureus	0.03720	0.03974	0.03758	0.03817
Salmonella chaleraeuis	0.02872	0.02976	0.02699	0.02849
Salmonella typhosa	0.02390	0.02338		0.02364
Shigella flexneri	0.02940	0.03014		0.02977

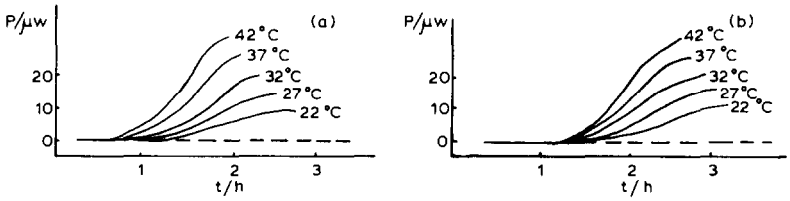

Fig. 6. Thermogenesis curves for bacterial growth in the log phase at different temperatures: (a) *Escherichia coli*, (b) *Shigella flexneri*.

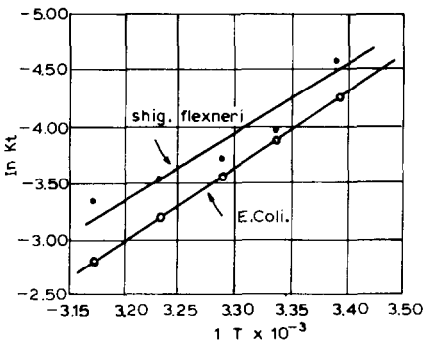

Fig. 7. Linear relation in k_t vs. T^{-1}.

TABLE 4

Rate constants of bacterial growth at various temperatures

Temperature T (°C)	k (min^{-1})	
	Escherichia coli	*Shigella flexneri*
22.00	0.01437	0.01017
27.00	0.02098	0.01938
32.00	0.02929	0.02552
37.00	0.03969	0.02977
42.00	0.05825	0.03050

TABLE 5

Results for the Arrhenius factors E_a and ln Z

Bacteria sample	E_a (kJ mol^{-1})	ln Z	r
Escherichia coli	53.09	17.40	0.9991
Shigella flexneri	40.88	12.78	0.9229

larly rate constants (k_t) were calculated at each temperature. These results indicate that ln k_t and $1/T$ are linearly proportional. So, with the help of the Arrhenius relation, the activation energy (E_a) and pre-exponential factor (Z) can be calculated, the results being shown in Figs. 6 and 7 and Tables 4 and 5.

CONCLUSIONS

(1) The experimental results indicate perfect thermograms have been determined. These reflect the changes of bacterial growth patterns (as the lag phase, exponential growth, stationary phase and decline phase of growth). For each bacterium highly characteristic and reproducible growth patterns can be observed under the same conditions, therefore these patterns can be used as a "fingerprint" to identify bacteria.

(2) These thermograms contain enough information about bio-thermokinetic metabolic processes to allow us to calculate the rate constant and activation energy parameters from these thermogenesis curves. This is very significant for microbiologists and bio-thermochemists.

(3) The accuracy of the rate constant obtained by this method can be checked as follows. The generation time (G) of a bacterium is a classic parameter of microbiology. It characterizes the multiplication rate of bacteria, for *Escherichia coli* culture on peptone at 37 °C $G = 17$ min [6]. This means that every 17 min the bacterial numbers double, so, using eqn. (2) when $n_t = 2n_0$ we obtain (G)

$$G = t = \ln 2/k \tag{6}$$

substituting $k = 0.03968$ (for *Escherichia coli*) in eqn. (2) we get $G = 17.46$ min.

This result conforms correctly to the literature value [6] and verifies the accuracy of the experiments.

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